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Prune-Belly Syndrome and Other Anomalies in a Stillborn Fetus With a Ring X Chromosome Lacking *XIST*

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Ring X chromosomes that lack the X inactivation center and fail to be inactivated have been implicated as a cause of mental retardation and multiple congenital anomalies. We report on a stillborn fetus with karyotype mos45,X/46,X,r(X) and early urethral obstruction or prune-belly sequence, single umbilical artery, limb deficiency, horseshoe kidney, cardiac hypertrophy, persistent left superior vena cava, and axial skeleton abnormalities. Fluorescent in situ hydridization (FISH) studies confirmed that the ring chromosome is X-derived and demonstrated that it lacks the *XIST* locus. The findings in this fetus are discussed with regard to the spectrum of phenotypes associated with monosomy X and small ring X chromosomes. Am. J. Med. Genet. 70:32–36, 1997.

KEY WORDS: X inactivation; *XIST;* ring X; prune-belly syndrome; Ullrich-Turner syndrome; VACTERL; mosaicism

INTRODUCTION

X inactivation is the mechanism that compensates for the sex difference in dosage of X-linked genes in mammals [Lyon, 1961]. A *cis*-acting locus on the long arm, termed the X-inactivation center (*XIC*), must be present for inactivation to occur [Brown et al., 1991b]. One gene in the *XIC* region, *XIST,* is transcribed only from the inactive X chromosome. Expression of the *XIST* gene is implicated in the initiation of X inactivation [Brown et al., 1991a]. Since terminal or interstitial deletions encompassing *XIC* are not observed in patients, functional disomy for large portions of the X chromosome is presumed to be lethal. Functional disomy of smaller regions can occur as a result of translocations [Schmidt and Du, 1992; Bardoni et al., 1993; Lahn et al., 1994] or duplications [Bardoni et al., 1994], and is associated with mental retardation and congenital anomalies.

Failure to dosage-compensate X-linked genes is hypothesized to explain the severe phenotype of some females with Ullrich-Turner syndrome (UTS) and mosaicism for a ring X chromosome, karyotype mos45,X/46,X,r(X) [Kushnick et al., 1987; Grompe et al., 1992; Van Dyke et al., 1992; Dennis et al., 1993; Cole et al., 1994; Collins et al., 1994; Migeon et al., 1993, 1994; Wolff et al., 1994]. Individuals with the 45,X/46,X,r(X) karyotype whose rings include the *XIC* locus generally show typical UTS anomalies such as short stature, amenorrhea, and minor abnormalities. By contrast, individuals with ring X chromosomes that lack the *XIC* locus or fail to express the *XIST* gene can display much more severe phenotypes atypical of UTS, including mental retardation, a Kabuki-like facies, and syndactyly or other limb defects [Migeon et al., 1993, 1994; Wolff et al., 1994].

We report autopsy findings and molecular cytogenetic studies on a 34-week-old stillborn fetus with karyotype 45,X/46,X,r(X) and multiple malformations. We demonstrate using FISH that the ring X chromosome lacks *XIST* sequences. To our knowledge, this is the first detailed phenotypic description of an abortus with a ring X that lacks *XIST.* These observations add to the spectrum of abnormalities associated with small ring X chromosomes.

CLINICAL REPORT

The fetus was stillborn to a 16-year-old primigravid African-American woman at 34 weeks of estimated gestation. The pregnancy was complicated by subclinical hepatitis B infection and two trichomonas infections treated with metronidazole. An oral glucose tolerance

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test was negative for gestational diabetes. There was no record of a sonogram. Family history indicated 2 miscarriages by the proposita's maternal grandmother.

The mother presented with spontaneous onset of labor. Sonography detected no fetal heart motion. The footling breech fetus was extracted with manual traction and weighed 2,070g (normal, 1,750 ± 494 g [Wigglesworth et al., 1991]). Crown-rump length was 29.8 cm (normal, 30.1 ± 3.5 cm [Wigglesworth et al., 1991]), and head circumference was 30.6 cm (normal, 31.5 cm [Stocker and Dehner, 1992]). At autopsy, external examination documented Potter face with apparently low-set ears and micrognathia, frontal bossing, massive abdominal distension, edematous ambiguous genitalia, imperforate anus, and distal left lower limb deficiency (Fig. 1). There were no hydrops, edema of the hands or feet, shield chest, or other obvious UTS signs, nor was there syndactyly. Upon internal examination, the urethra was stenotic but microscopically patent throughout its course, and there was massive dilatation and hypertrophy of the bladder, severe bilateral hydroureter, hydronephrosis, displacement of numerous abdominal organs, attenuation and fibrosis of the abdominal wall musculature, and marked pulmonary hypoplasia, all consequences of the early urethral obstruction or prune-belly sequence (Fig. 2). Dissection of the cardiovascular system showed a persistent left superior vena cava draining into a right coronary sinus, a single umbilical artery arising from the left internal iliac artery, and enlargement of all four chambers of the heart unaccompanied by valvular dilatation or malformation, aortic coarctation, or septal defects. A horseshoe kidney, atresia of the right distal ureter, colovesical fistula, and hepatosplenomegaly were also present. A uterus and streak ovaries were identified and confirmed histologically. Radiographic studies confirmed the lower limb abnormalities seen on external examination and demonstrated thoracic butterfly vertebrae, lumbosacral vertebral fusion segmentation anomaly, and 14 left ribs. Histologic sections of the liver showed no evidence of hepatocyte necrosis or he-

Fig. 1. Lateral view of the fetus, demonstrating Potter face, frontal bossing, massive abdominal distension, and distal left lower limb reduction.

Fig. 2. Single left umbilical artery (small arrow) courses to the umbilicus around the massively distended bladder, which occupies most of the abdominopelvic cavity. The left lower limb defect likely resulted from ischemia during development, due perhaps to compression of the external iliac artery by the enlarging bladder, a vascular steal phenomenon in which the single umbilical artery shunted blood away from the external iliac artery, or to a combination of these mechanisms. The umbilical vein (large arrow) is stretched over the dome of the bladder as it courses from the umbilicus to the liver. The miniscule thoracic cavity reveals pulmonary hypoplasia, with lungs barely discernible, and cardiomegaly.

patic fibrosis, and no hepatitis B virus was isolated from cultured liver tissue.

CYTOGENETIC AND MOLECULAR ANALYSIS

Routine chromosome analysis was performed on PHA-stimulated peripheral blood leukocytes. G-banding of 20 cells showed the karyotype mos45,X[7 cells]/46,X,r(?X)[13 cells] (Fig. 3). FISH studies were performed on metaphase spreads from cultured kidney fibroblasts, using standard techniques [Knoll and Lichter, 1995]. Monochromatic images were captured using an Axiophot microscope (Carl Zeiss, Oberkochen, Germany) equipped with a cooled charge-coupled device camera (Photometrics Ltd., Tucson, AZ), and were pseudocolored using Adobe Photoshop. Two-color FISH images were recorded with Ektachrome 400 film. Chromosomes were counterstained with DAPI. The ring chromosome hybridized to an X centromere probe, pBamX7 [Durfy and Willard, 1987] (Fig. 4a), confirming its X origin. In some metaphases the ring was larger and showed two discrete regions of hybridization to the centromere probe (Fig. 4b), consistent with a double ring arising via mitotic instability. Two-color FISH was performed to determine whether the ring X contained the *XIST* locus. The X centromere was detected using digoxigenin-labeled pBamX7 probe with Texas red as the fluorophore. *XIST* sequences were concomitantly detected using a biotinylated cosmid probe (ICRFc100H0130) [Brown et al., 1992], with fluorescein isothiocyanate as the fluorophore. This cosmid probe routinely detects both *XIST* loci in >80% of metaphases from normal females (not shown). Two metaphases demonstrating hybrization of the *XIST* probe to the normal X but not the ring X chromosome from the stillborn fetus are shown (Fig. 4c,d). The same result was observed in >5 additional metaphases, and only one *XIST* signal was present in each of >10 interphase nuclei with two X centromere signals (not shown). Additional studies were hampered by senescence of the fetal cells.

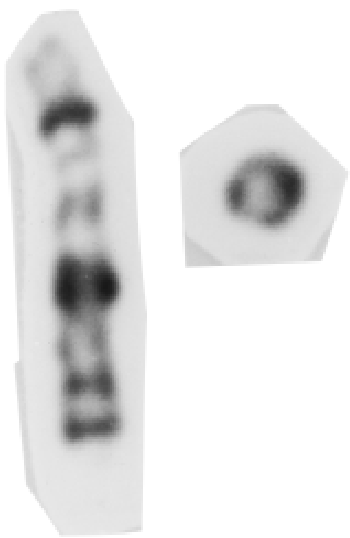

Fig. 3. G-banded normal X and ring X chromosomes from the fetus.

DISCUSSION

The autopsy showed multiple gross malformations and histologic findings which were consistent with early urethral outlet obstruction leading to prune-belly syndrome (PBS) and oligohydramnios sequence. Renal anomalies such as horseshoe kidney, unilateral renal agenesis, and duplicated renal pelvis are common in UTS, but PBS is infrequent in patients with monosomy X. Pagon et al. [1979] cited a single case of PBS in a UTS infant; they mentioned no evidence of urinary tract obstruction and instead attributed the abdominal distension to fetal ascites secondary to impaired lymphatic development. Lubinsky et al. [1980] described two additional cases. One was a 3-year-old girl with a nonmosaic 45,X karyotype, many classic signs of UTS, absence of the oblique abdominal muscles, presence of the rectus abdominis muscles, normal genitalia, and no firm evidence of urologic tract malformation. The other was an infant who died at 11 days of life with pyelographic evidence of right megaloureter and a small left kidney, but no radiographic evidence of obstructive renal changes. Autopsy findings were not described for this case. Savanelli et al. [1986] reported a 45,X stillborn infant with hypoplastic abdominal muscles, horseshoe kidney, "atrophy" of the left ureter at the ureteropelvic junction, atrophy and cystic change of the left kidney, coarctation of the aorta, and a large bladder, but with a normal urethra. We could find no previous report of PBS in a 45,X/46,X,r(X) mosaic. It is possible that a ring X was present but not detected in previous cases of UTS with PBS due to the extremely small size of some rings or to mosaicism and failure to sample cells containing the ring X in tissues examined.

Additional anomalies in the fetus we studied, as in the heart and axial skeleton, are not clearly related to early urethral obstruction or oligohydramnios and are also atypical of UTS. Cardiac abnormalities associated with monosomy X include aortic coarctation and bicuspid aortic valve, neither of which was present in the fetus. The usual skeletal manifestations of UTS include cubitus valgus, short fourth or fifth metacarpals, short cervical vertebrae, scoliosis, and congenital hip dislocation.

The constellation of vertebral anomalies, minor cardiac defects, single umbilical artery, renal anomalies, and imperforate anus in this fetus warrants a consideration of the VACTERL association (vertebral anomalies, anal atresia, congenital cardiac disease, tracheoesophageal fistula, renal anomalies, radial dysplasia, and other limb defects). The VACTERL association is not characterized by an abnormal karyotype. A possible relationship between the VACTERL association and PBS is suggested by the report by Reinberg et al. [1991] of 7 females with PBS. One of their cases had the VATER (vertebral anomalies, anal atresia, tracheoesophageal fistula, renal anomalies, radial dysplasia) association; another 2 had imperforate anus. All 7 had the 46,XX karyotype. Interestingly, two pedigrees have been described in which the VACTERL association and hydrocephalus appear to show X-linked inheritance [Genuardi et al., 1993; Wang et al., 1993], implicating

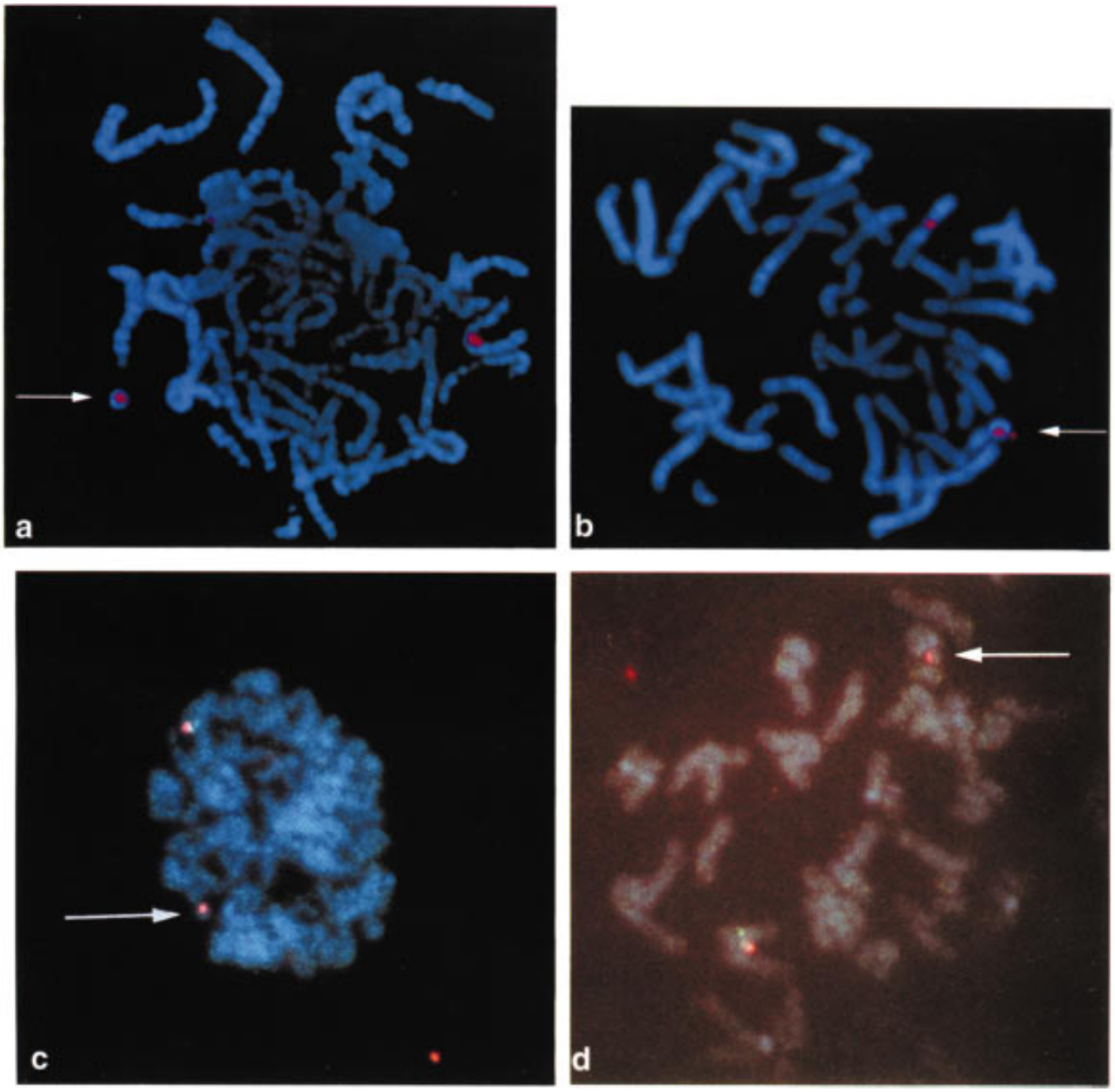

Fig. 4. **a,b:** FISH with digoxigenin-labeled X centromere probe. **c,d:** Two-color FISH with digoxigenin-labeled X centromere probe (red) and biotinylated *XIST* probe (green). Arrows indicate hybridization to ring chromosomes.

abnormal expression of genes encoded by the X chromosome in the cause of some cases.

We cannot exclude the possibility that the ring X and the phenotype of this fetus are coincidental. Other considerations in this case include prenatal exposure to metronidazole or hepatitis B virus. However, there is no evidence that metronidazole increases risk of birth defects [Piper et al., 1993]. There was also no evidence of hepatitis in the fetus, nor were any of the congenital malformations specifically associated with hepatitis B infection. We therefore favor the presence of mosaicism for a ring X chromosome lacking *XIC,* and consequent functional disomy for pericentromeric X-linked genes, to explain the unusually severe and atypical findings in this fetus. The high proportion of cells carrying the ring chromosome (65%), at least in the tissues evaluated cytogenetically, may have contributed to the severity of the phenotype. Mosaicism for a ring X chromosome should be considered in other fetuses or infants with PBS or VACTERL-like abnormalities. Detailed molecular analysis of the ring X chromosome in future cases may help to identify disomic genes responsible for the phenotype.

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